

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1 (Original) A method for evolving a protein encoded by a DNA substrate molecule comprising:

- (a) digesting at least a first and second DNA substrate molecule, wherein the at least a first and second substrate molecules differ from each other in at least one nucleotide, with a restriction endonuclease;
- (b) ligating the mixture to generate a library of recombinant DNA molecules;
- (c) screening or selecting the products of (b) for a desired property; and
- (d) recovering a recombinant DNA substrate molecule encoding an evolved protein.

Claim 2 (Original) The method of claim 1, wherein the restriction endonuclease generates non-palindromic ends at cleavage sites.

Claim 3 (Original) The method of claim 1, wherein the substrate molecules have been engineered to contain at least one recognition site for a restriction endonuclease having non-palindromic ends at cleavage sites.

Claim 4 (Original) The method of claim 1, wherein (a) – (d) are repeated.

Claim 5 (Original) The method of claim 1, wherein the DNA substrate molecule comprises a gene cluster.

Claim 6 (Original) The method of claim 1, wherein at least one restriction endonuclease fragment from a DNA substrate molecule is isolated and subjected to mutagenesis to generate a library of mutant fragments.

Claim 7 (Currently Amended) The method of ~~step~~ claim 6, wherein the library of mutant fragments is used in the ligation of (b).

Claim 8 (Original) The method of claim 7, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

Claim 9 (Original) The method of claim 6, wherein mutagenesis comprises recursive sequence recombination.

Claim 10 (Original) The method of claim 1, wherein the products of (d) are subjected to mutagenesis.

Claim 11 (Original) The method of claim 10, wherein mutagenesis comprises recursive sequence recombination.

Claim 12 (Original) The method of claim 1, wherein the products of (d) are used as a DNA substrate molecule in (b).

Claim 13 (Original) The method of claim 10, wherein the products of claim 10 are used in (d).

Claim 14 (Original) The method of claim 1, wherein the recombinant DNA substrate molecule of (d) comprises a library of recombinant DNA substrate molecules.

Claim 15 (Original) An evolved protein produced by the method of claim 1.

Claims 16-273 (Cancelled).

Claim 274 (New) A method of producing a progeny library comprised of chimerized but pre-determined polynucleotide sequences each of which is comprised of a pre-determined number of building block sequences that are assembled in non-random order, the method comprising:

generating a plurality of pre-determined nucleic acid building block sequences obtained from polynucleotide sequences that encode enzymes or fragments thereof and comprised of sequences delineated by demarcation points selected from aligned progenitor sequences; and

non-stochastically reassembling said nucleic acid building block sequences to produce said chimerized but pre-determined polynucleotide sequences, such that a designed overall assembly order is achieved for each of said chimerized but pre-determined polynucleotide sequence.

Claim 275 (New) A method of producing a library comprised of chimerized but defined polynucleotide sequences each of which is comprised of a defined number of polynucleotide segments that are assembled in an ordered fashion, the method comprising:

a) generating a plurality of defined polynucleotide segments of substrate nucleic acid sequences
that encode full-length enzymes,
and wherein the borders defining the polynucleotide segments are selected from the aligned substrate nucleic acid sequences; and

reassembling said defined polynucleotide segments in order
thereby producing the library of chimerized but defined polynucleotide
sequences,

such that said segments are reassembled in an ordered fashion
for each chimerized but defined polynucleotide sequences encoding full-
length enzymes.